Exploring olfactory dysfunction and lysosomal mutations in the prodromal α-synuclein preformed fibrils mouse model

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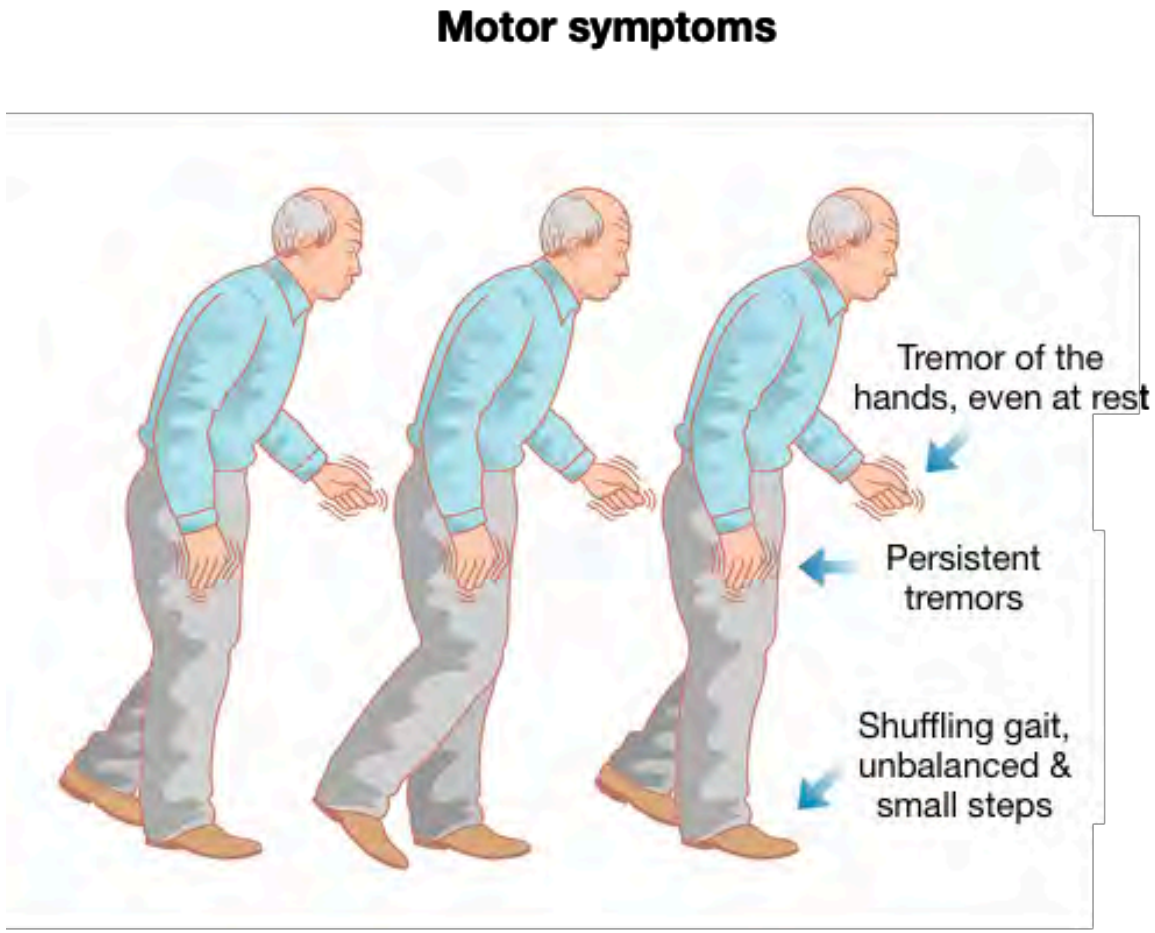
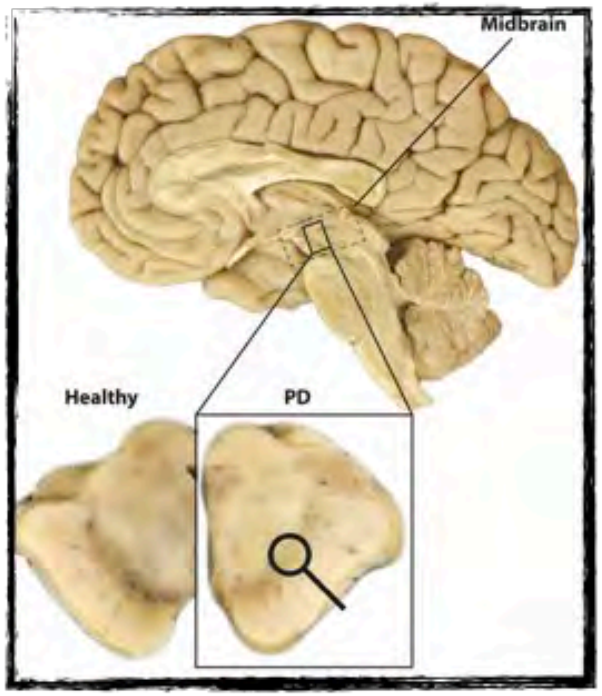
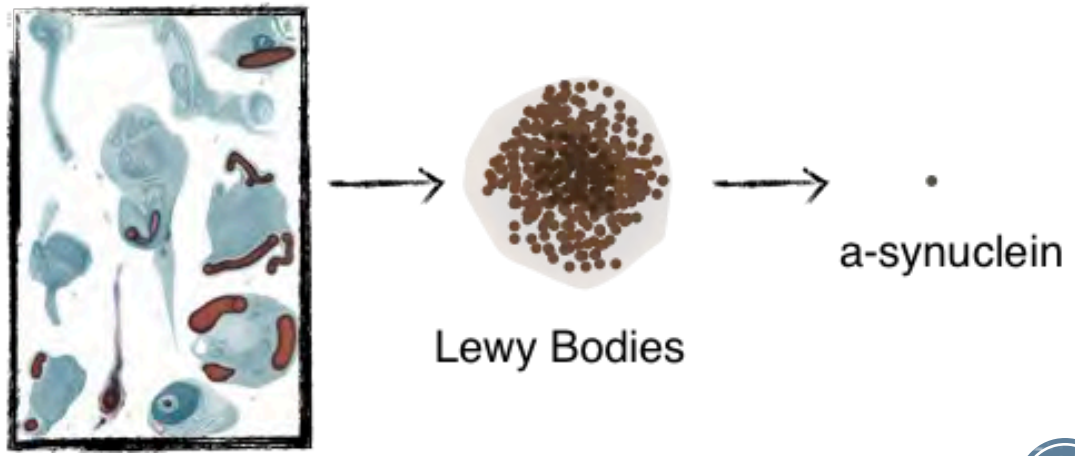
UniSA Clinical & Health Sciences
Health and Biomedical Innovation
Research Seminars 2021

Parkinson’s disease (PD)







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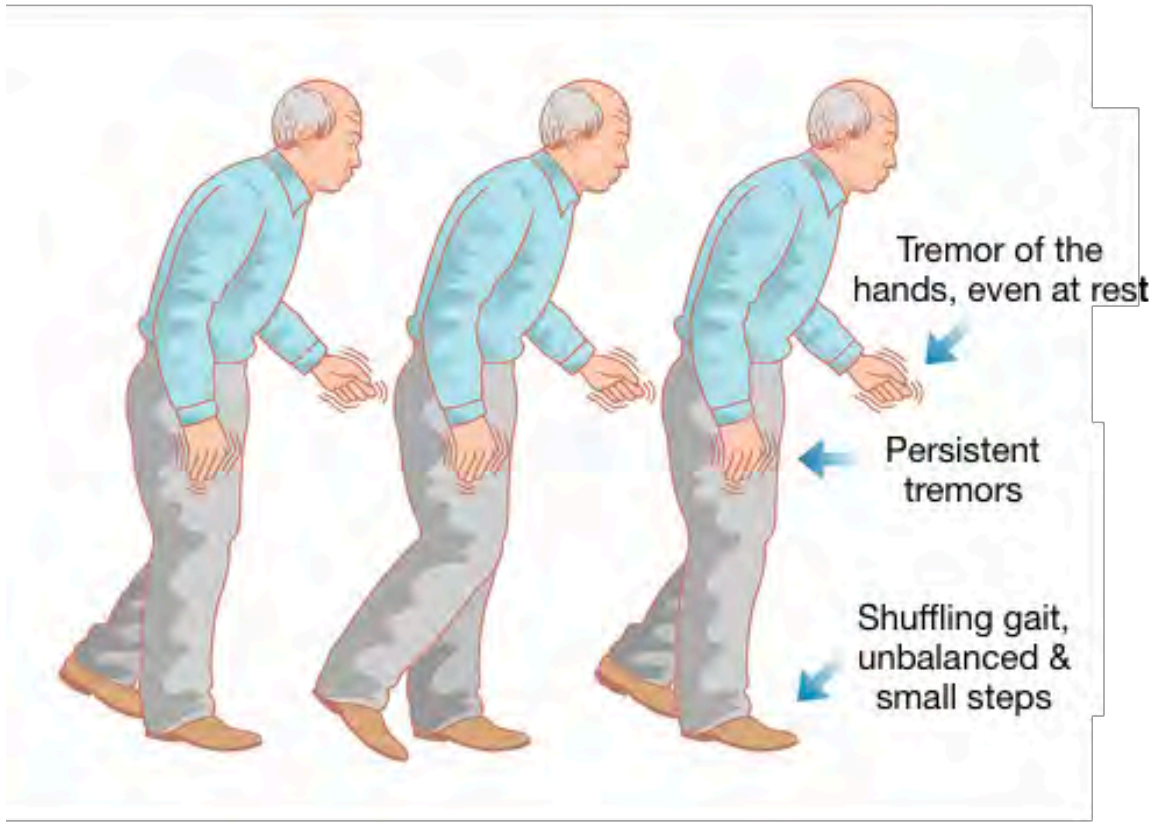
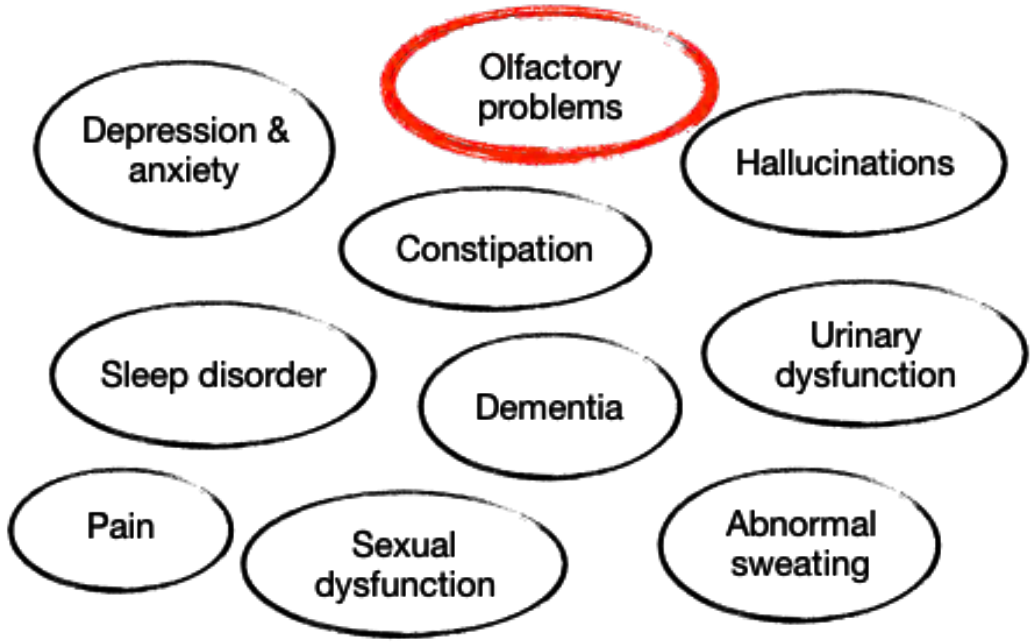
Non-motor/prodromal Parkinson's disease (PD)

Motor symptoms



Non-motor symptoms



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Hyposmia (olfactory deficits)

Over 90% of people with PD

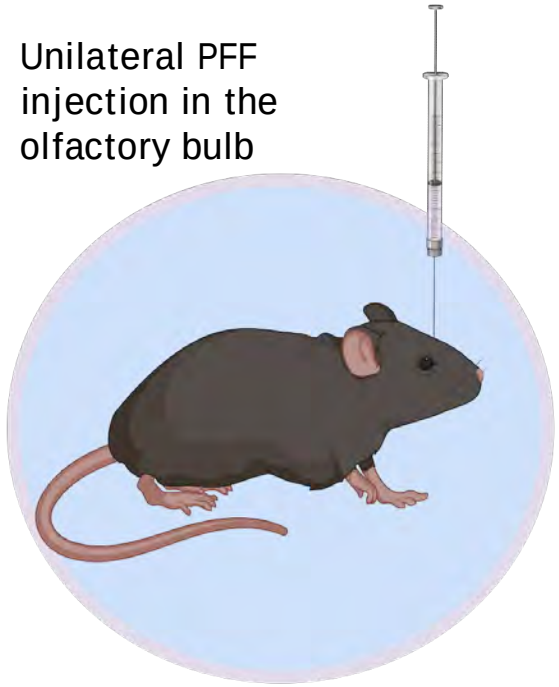
Olfactory dysfunction can occur up to 10 years before the onset of motor symptoms

Braak's staging, based on the analysis of post-mortem tissue from PD patients, hypothesizes α-synuclein pathology is initially present in the dorsal motor nucleus of the vagus, as well as the olfactory bulb (OB) and the anterior olfactory nucleus (AON)

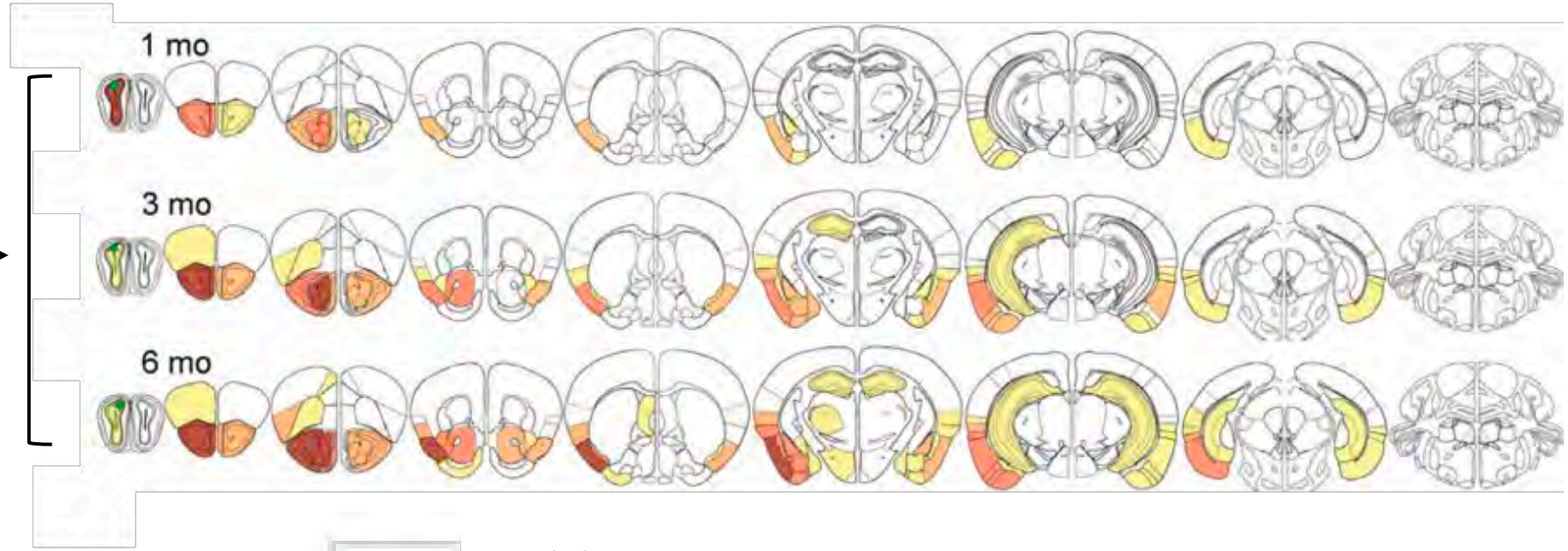
Thus, the olfactory bulb appears to be one of the earliest regions affected in disease

Prodromal PD α-synuclein preformed fibrils (PFF) mouse model

Unilateral PFF injection in the olfactory bulb



pSer129 positive α-synuclein pathology spreads to connected brain regions over time



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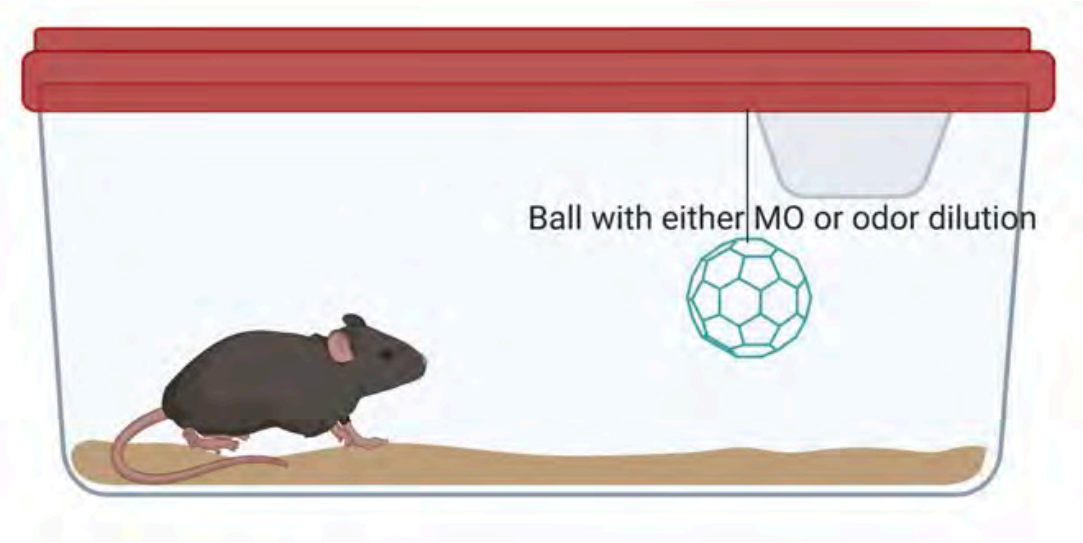
Rey et al. (2016) JEM

Prodromal PD a-synuclein preformed fibrils (PFF) mouse model

Assessed odor discrimination, odor retention and odor detection deficits

Olfaction was investigated using an odor threshold test:
•Mouse placed in clean cage with ball containing either mineral oil (MO) or diluted odor

•Time during the 50s trial that the mouse spent with nose placed within 1 cm from the ball was recorded



Test day exposure pattern: MO -> MO -> MO ->
Most diluted odor -> Less diluted odor -> Least diluted odor

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Rey et al. (2016) JEM

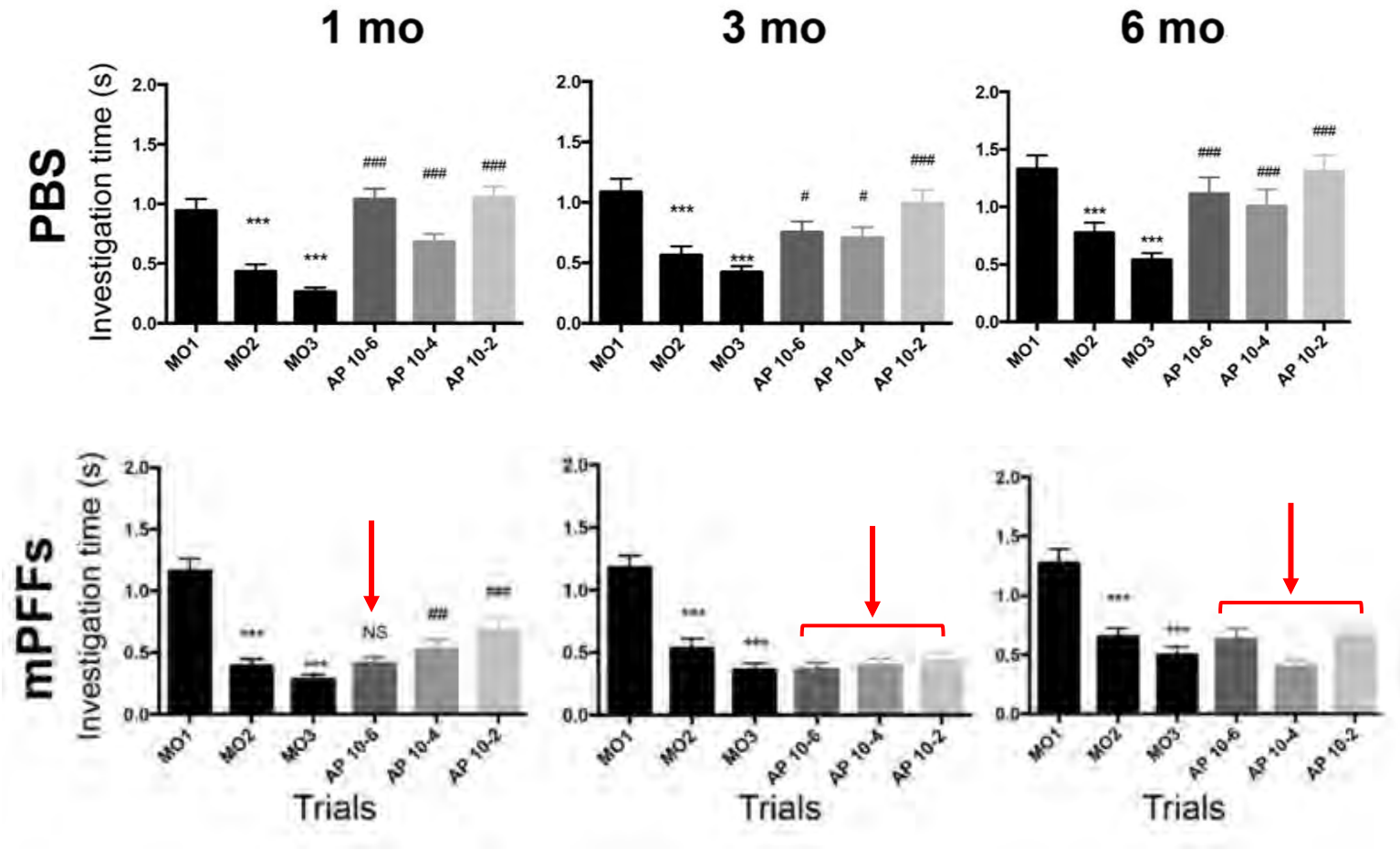
Prodromal PD a-synuclein preformed fibrils (PFF) mouse model

As the mice get acclimated with MO odor, they sniff less for each exposure

Mice sniffs more when exposed to all dilutions of odor compared to the last MO

PFF injected mice have minor deficit at 1-month

At 3 and 6 months, the PFF injected mice don't detect any of the odor dilutions



n= 25-30 female mice per group tested

Rey et al. (2016) JEM

Prodromal PD a-synuclein preformed fibrils (PFF) mouse model

Other factors than just olfaction may impact outcome:

- Ability to move (many PD models have impaired movement)

- Anxiety and depression

- Exploratory/food seeking behavior (many olfactory tests are food based)

- The researcher's interpretation of animal behavior and reflexes for timing

—> Issues with reproducibility between labs and even within labs

Thus, our aim was to optimize a more objective olfactory test to use in PD models

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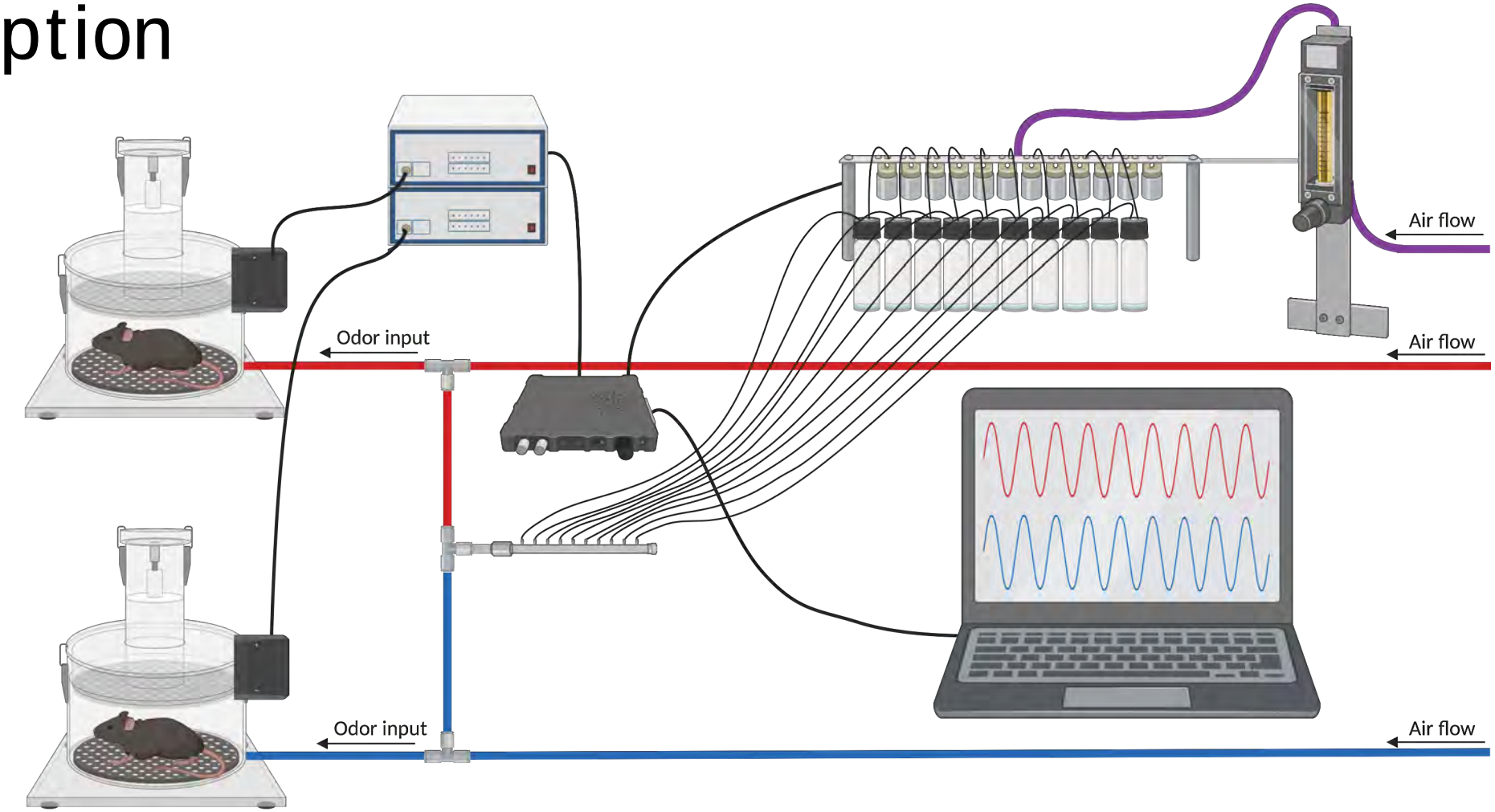
Rey et al. (2016) JEM

Semi-automated system for assaying mouse odor perception

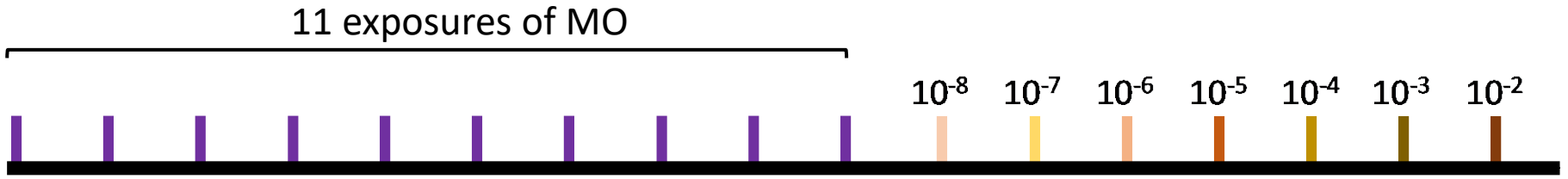


Johnson et al. (2020) Sci Rep

Our new olfactory testing approach- protocol

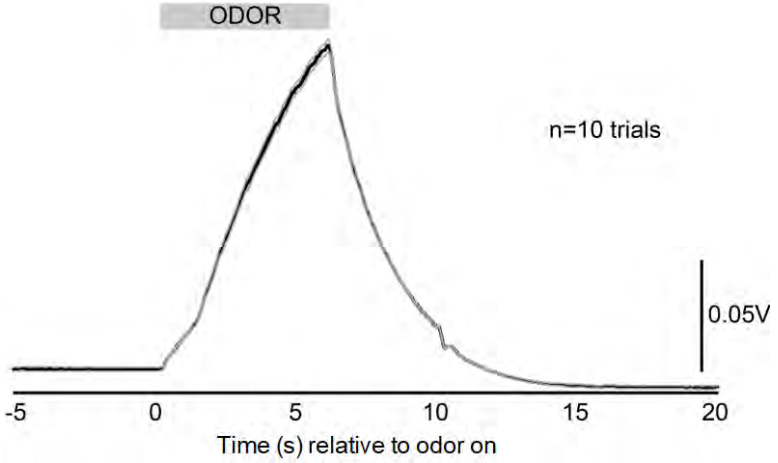
1) Habituation:
For 3 days prior to testing, the mice are put in the chambers for 30 min and exposed to MO every min

2) Testing: 11 exposures of MO every min, followed by the odor dilutions ranging from 10^{-8} to 10^{-2}



Mice are tested for 3 consecutive days using 3 different odors,
all diluted to 2 Torr (normalizing vapor pressure) prior dilution series

Photoionization detector →
- average traces of 10 trials



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Johnson et al. (2020) Sci Rep

Our new olfactory testing approach- analysis

We define “Investigatory sniffing” as anything above 6Hz (threshold)
—> We compare time spent engaged in investigatory sniffing between different groups

n

Mouse responding to odor

Mouse not responding to odor

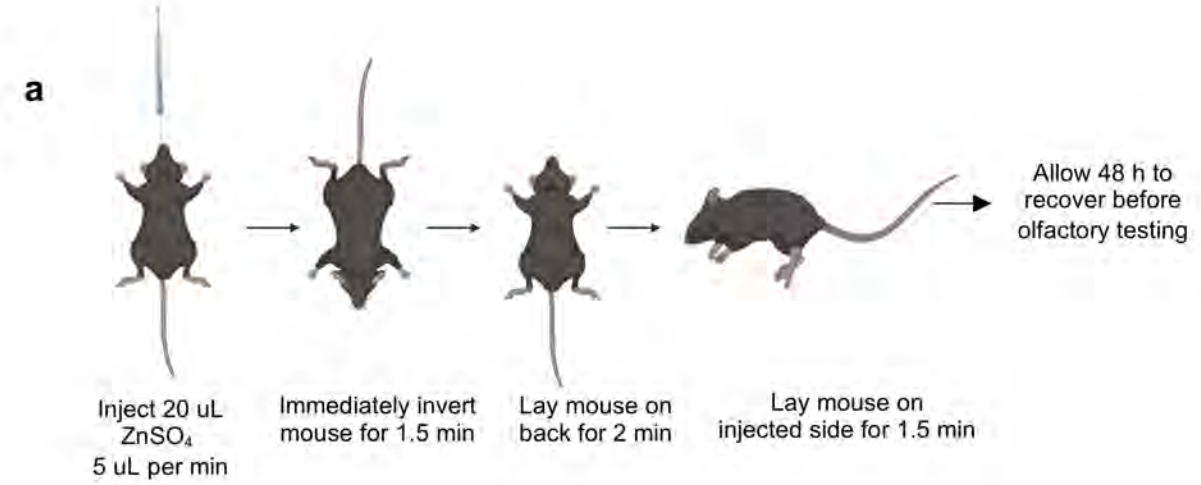
McAfee et al., (2016) J Neurosci Methods
Wesson et al., (2008) Chem Senses
Johnson et al. (2020) Sci Rep

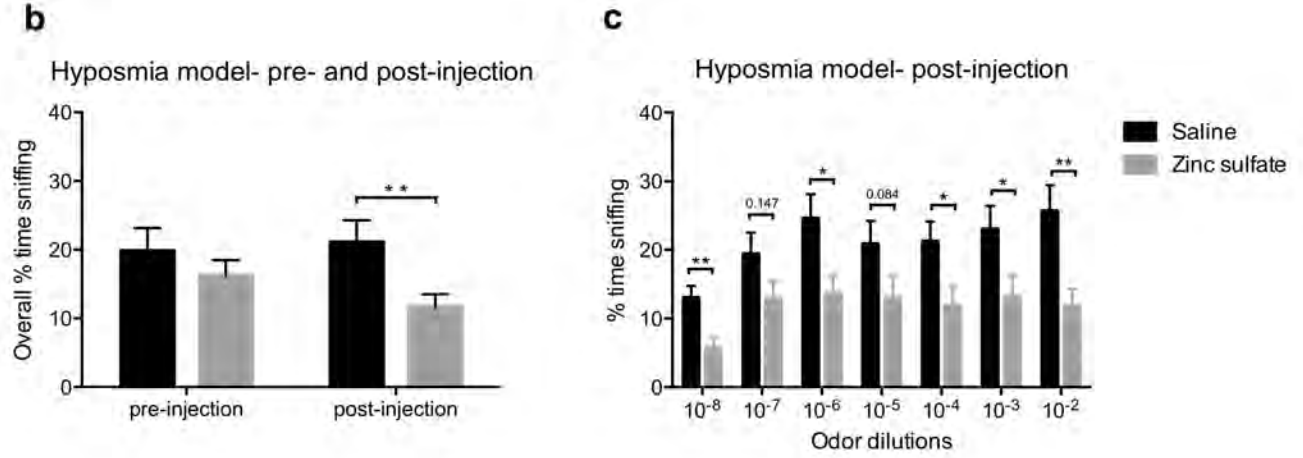
Our new olfactory testing approach- validation

Bilateral intranasal zinc sulfate model of acute hyposmia

- Destroys olfactory neurons in the olfactory epithelium

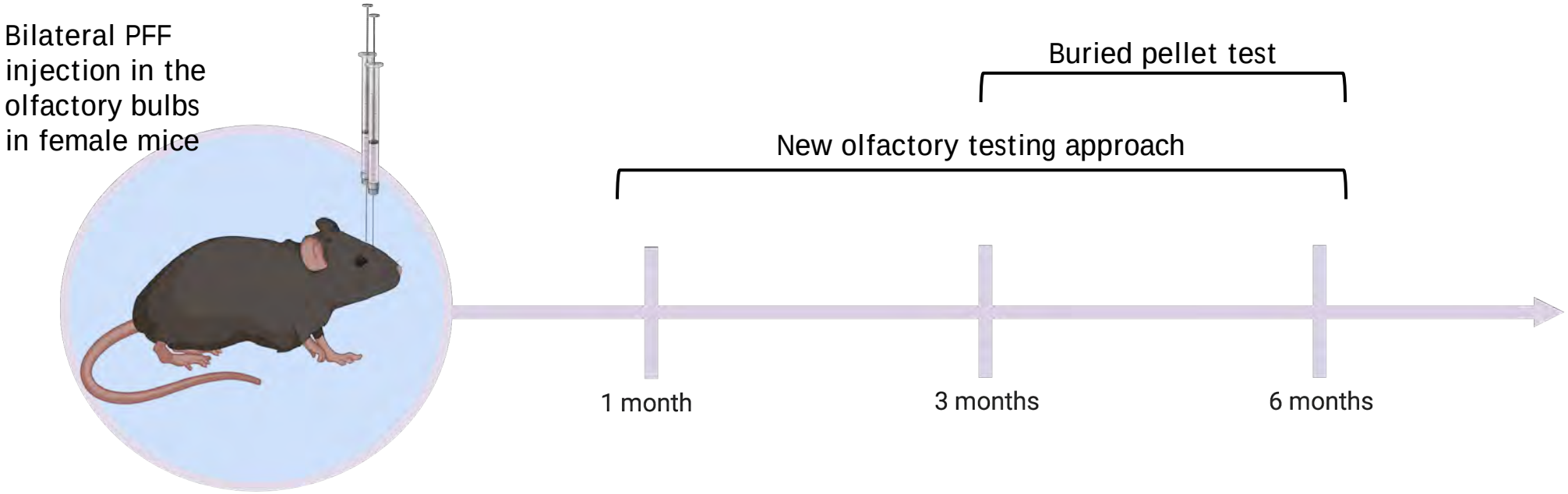
Shows our new olfactory testing approach is capable of detecting olfactory impairments





Ducray et al. (2002) Eur J Neurosci
Johnson et al. (2020) Sci Rep

Our new olfactory testing approach- PFF prodromal PD model



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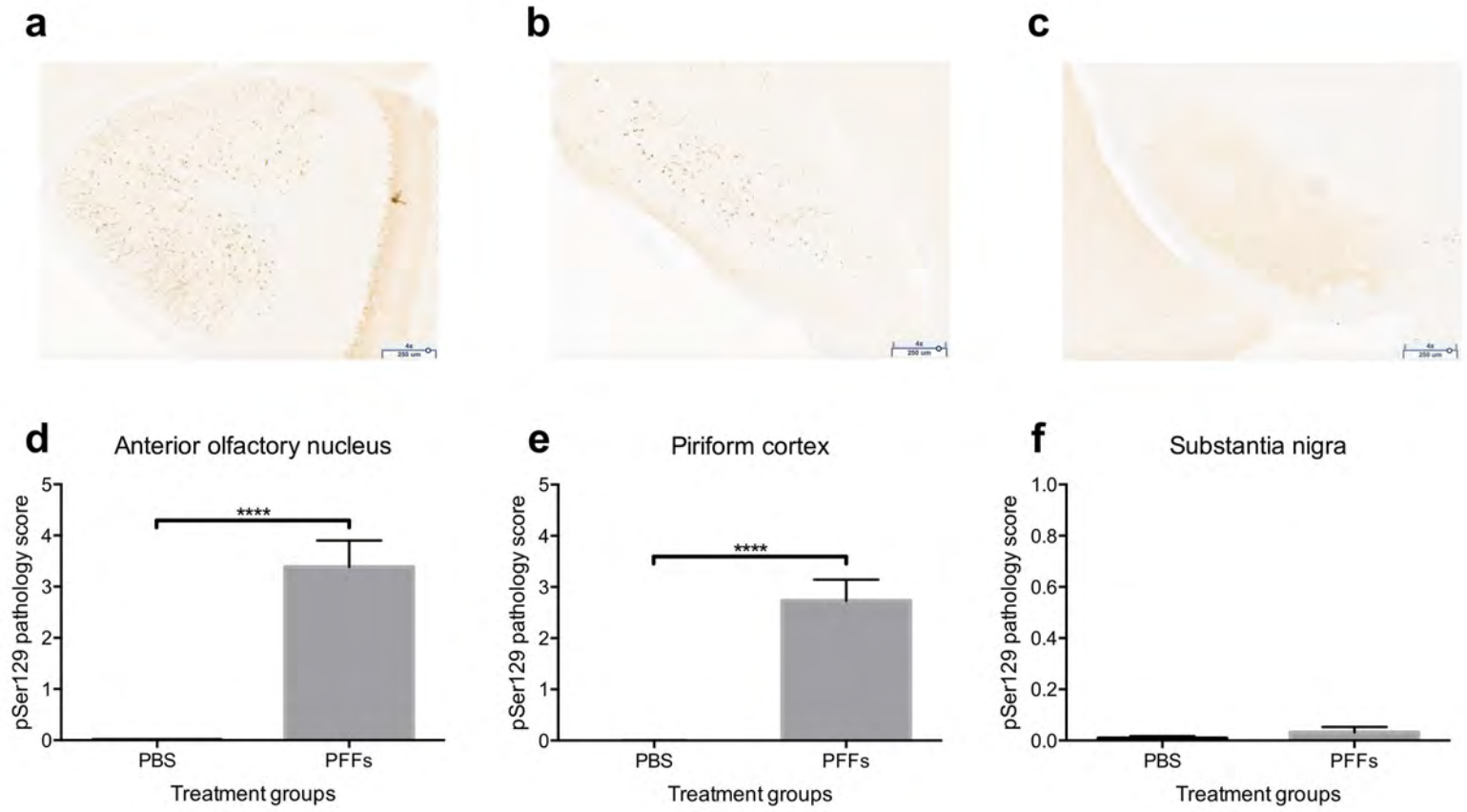
Johnson et al. (2020) Sci Rep

pSer129 α-synuclein load - PFF prodromal PD model

Extensive accumulation of phosphorylated α-synuclein in the AON and PCx, both are secondary olfactory structures

No pathology in the SN at 6-months post-injection



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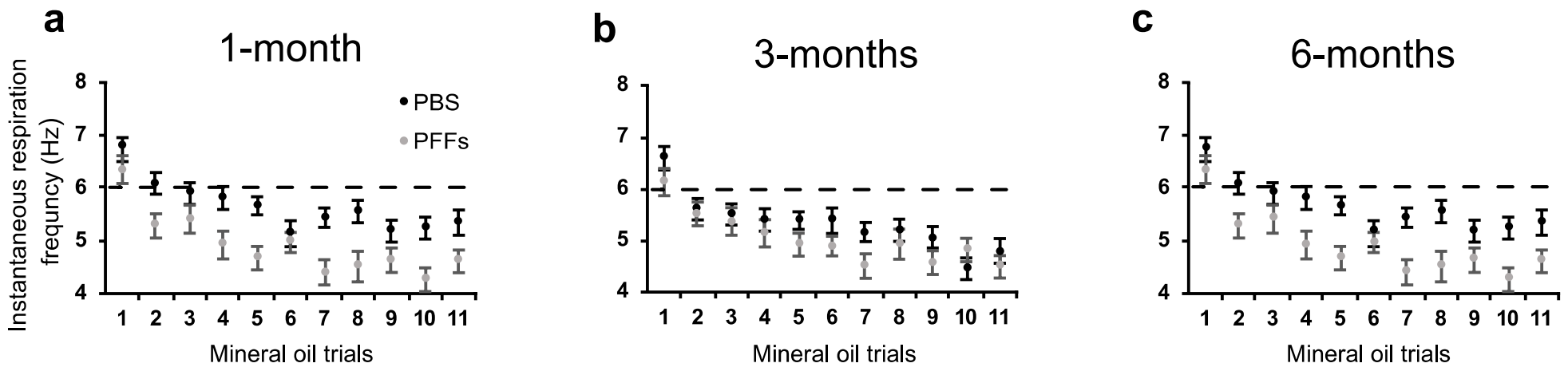
Habituation - PFF prodromal PD model



Both PBS and PFF-injected female mice can engage in investigatory sniffing and acclimate
- evident by reduced response to MO over multiple exposures

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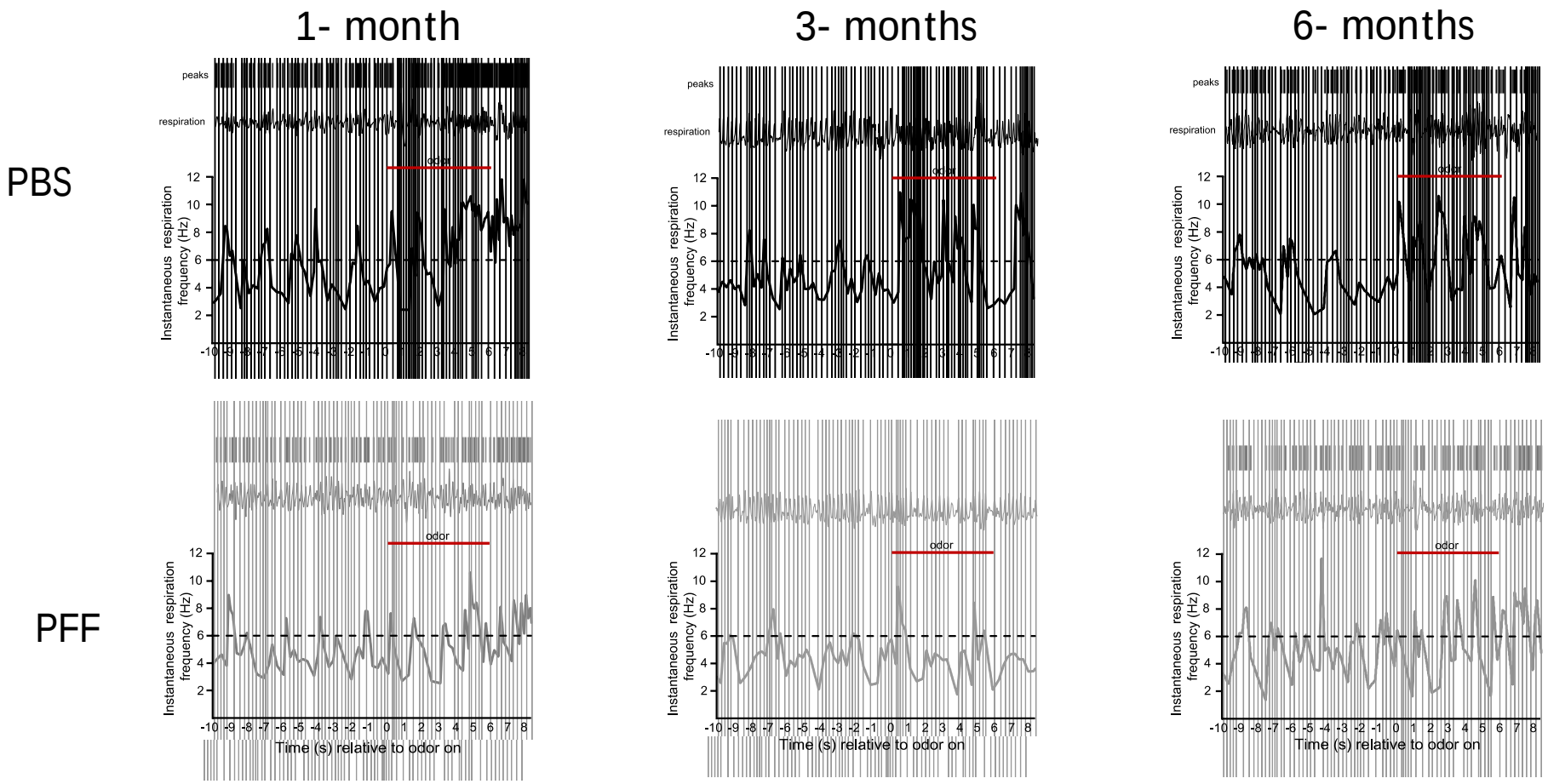
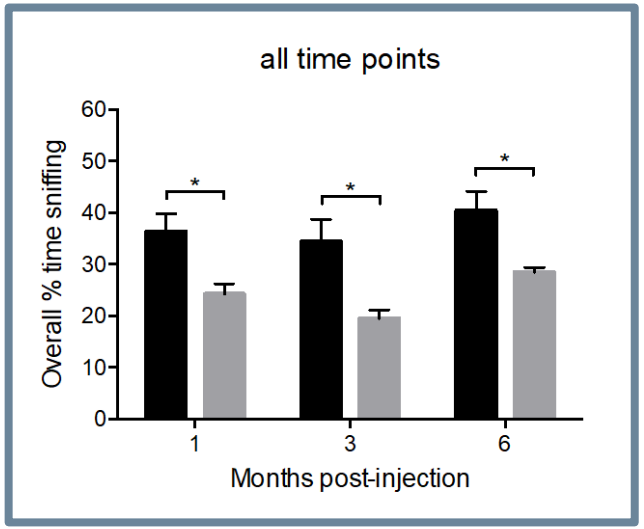
Johnson et al. (2020) Sci Rep

Respiration traces - PFF prodromal PD model



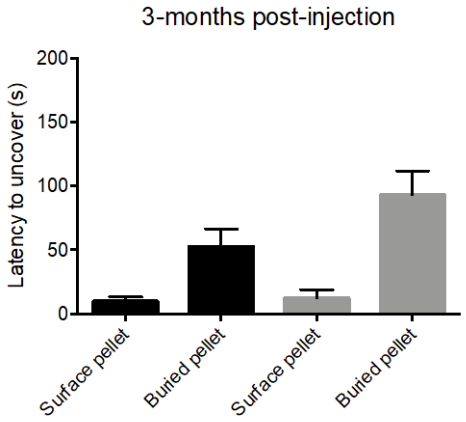
Johnson et al. (2020) Sci Rep

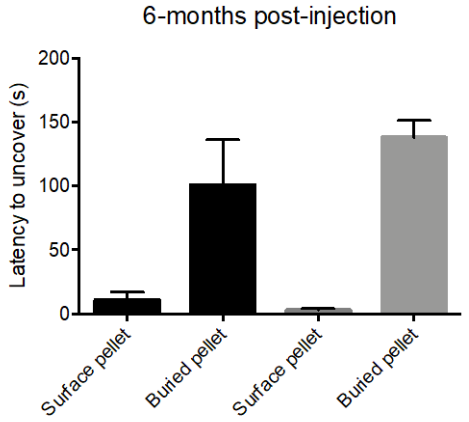
Olfactory testing results - PFF prodromal PD model



Buried pellet test

- Mice fasted overnight
- Pellet/treat (Cap'n Crunch) hidden under bedding in clean cage
- Time taken for mouse to uncover treat recorded (average of 3 trials)





PFF-injected female mice engage in less investigatory sniffing than PBS-injected

No difference when evaluated with buried pellet test

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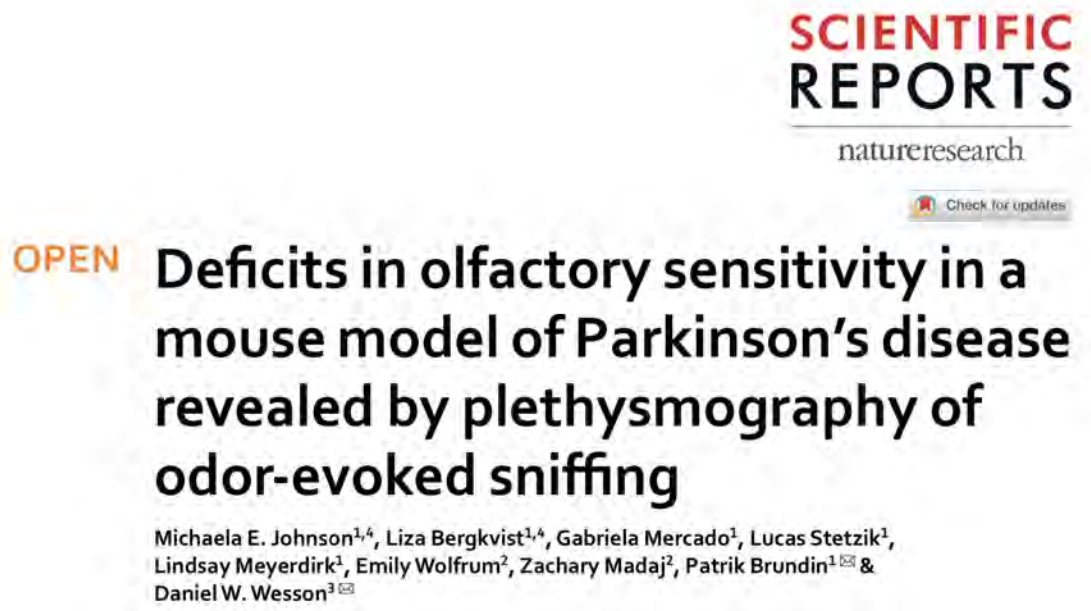
Johnson et al. (2020) Sci Rep

Summary

- Our semi-automated system appears more sensitive, and therefore capable of detecting smaller changes in olfactory perception that may go undetected using manual testing.
    - only require 5-6 animals per group to detect differences.

- Deficit in investigatory sniffing in PFFs-treated female mice is likely due to an olfactory sensory deficit rather than a deficit in the motor act of sniffing itself, as no pathology in SN or obvious signs of motor impairments.

- Can be used for the development and evaluation of potential therapeutic interventions aimed to prevent olfactory deficits and the spreading of a-synuclein pathology.



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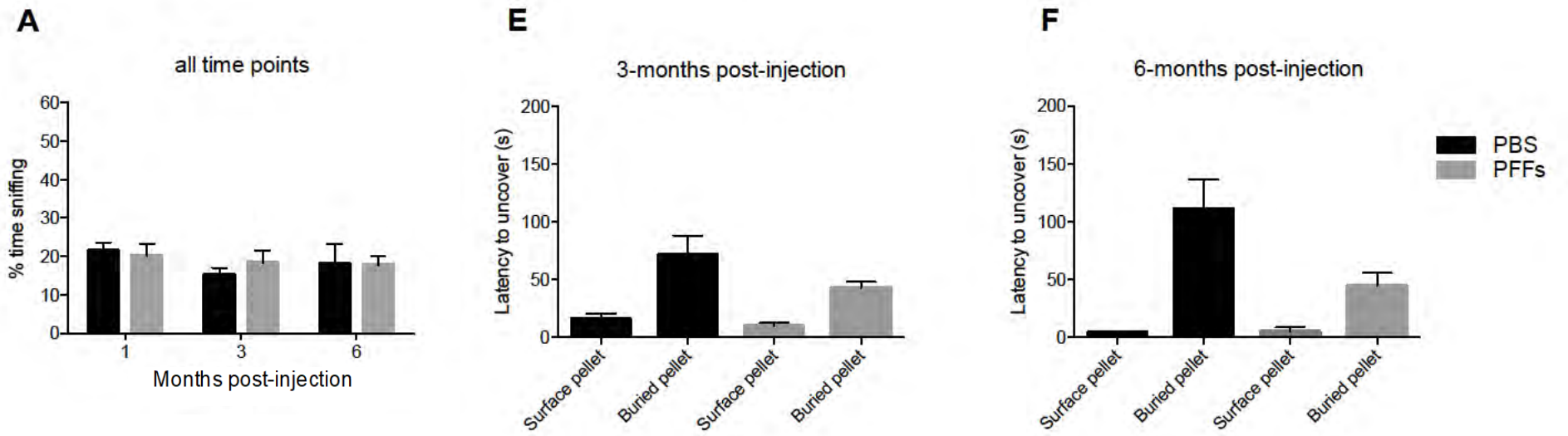
Male mice don’t show this olfactory deficit



- Despite having similar a-synuclein pathology loads in the anterior olfactory nucleus and piriform cortex we did not see this phenotype in male mice
  - *n*= 5-6 per group

- Further investigation into how sex and/or gonadal hormones influence odor perception in this model is needed

SCIENTIFIC REPORTS
nature research

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OPEN

Deficits in olfactory sensitivity in a mouse model of Parkinson’s disease revealed by plethysmography of odor-evoked sniffing

Michaela E. Johnson[1,4], Liza Bergkvist[1,4], Gabriela Mercado[1], Lucas Stetzik[1], Lindsay Meyerdirk[1], Emily Wolfrum[2], Zachary Madaj[2], Patrik Brundin[1] & Daniel W. Wesson[3]

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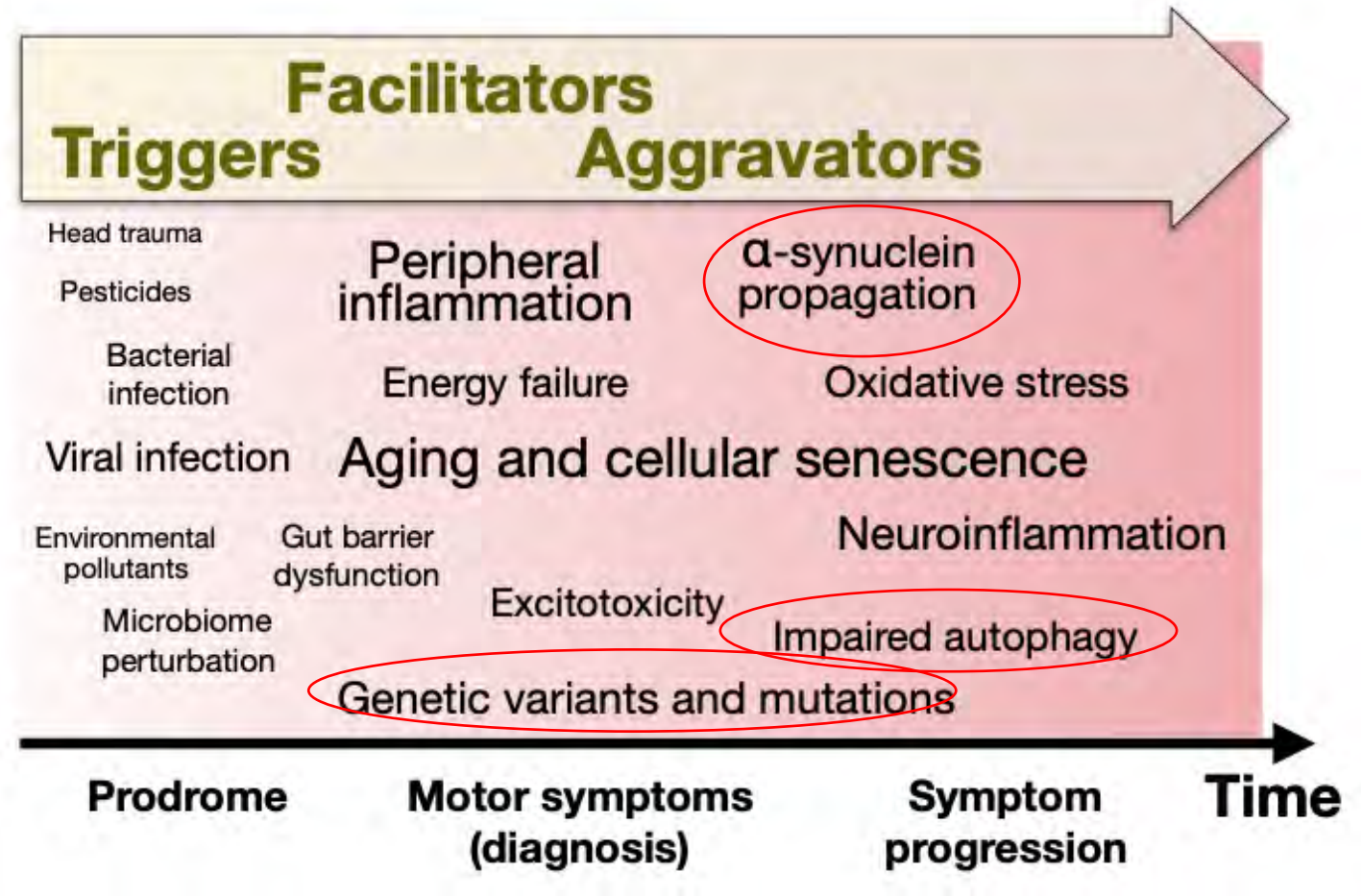
PD pathogenesis

- The exact cause of sporadic PD (90%) remains unknown
  - previous gene-environment and multi-hit hypotheses
- There is evidence for many factors playing a role in PD pathogenesis
- We proposed a new hypothesis that divides these factors into three categories: triggers, facilitators, and aggravators.
- We think that each of these factors play distinct roles at different stages of the disease.

PD pathogenesis

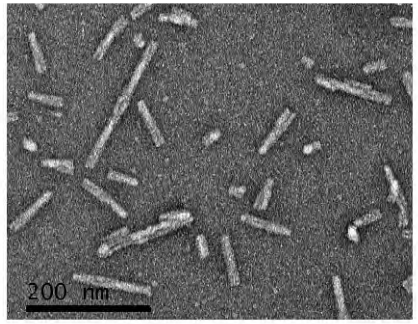
Vast array of combinations of factors in the three categories can explain variability observed in patients with PD

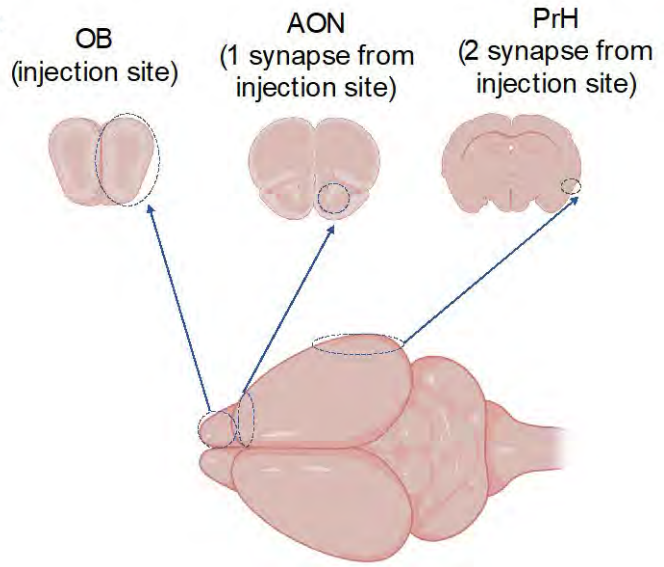


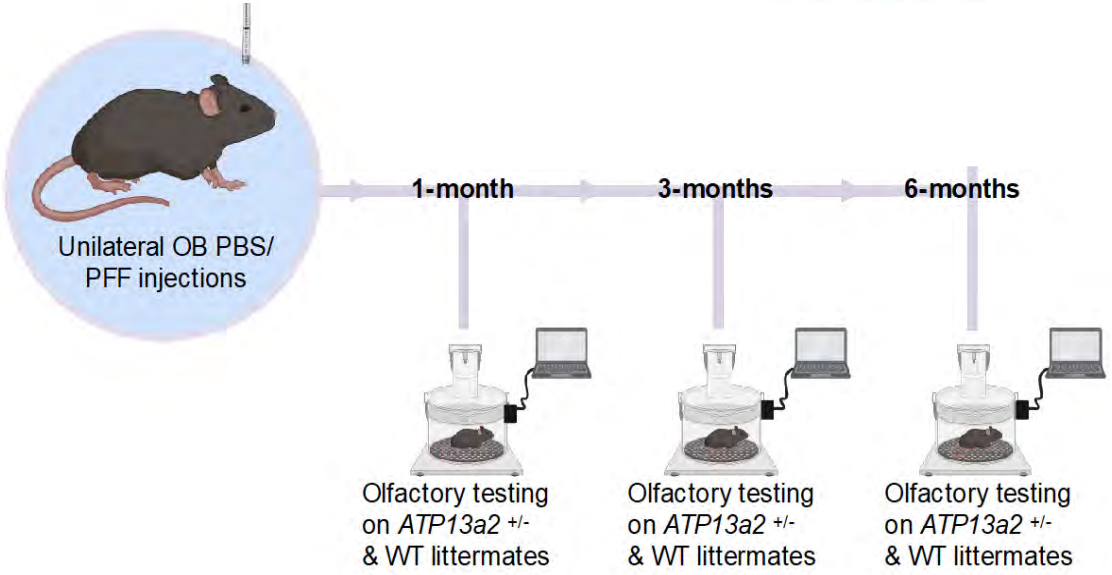
PARK9 (*ATP13a2)*

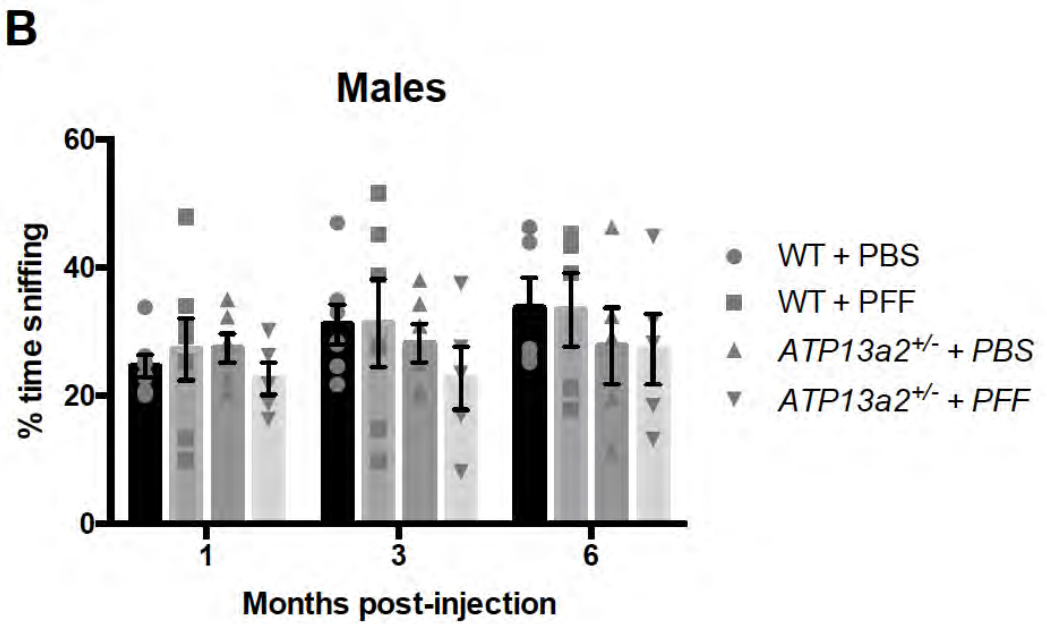
- PARK9 (*ATP13Aa2)* homozygous loss-of-function or compound heterozygous mutations cause Kufor-Rakeb syndrome, a rare genetic form of juvenile-onset parkinsonism
- Heterozygous carriers have an increased risk of developing early-onset sporadic PD
- *PARK9* gene encodes a lysosomal P5-type ATPase, physiological function is not completely understood
- Contribute to PD by reducing lysosomal degradation of α-synuclein, affecting externalization of α-synuclein via exosomes, and Zn^{2+} dyshomeostasis leading to mitochondrial dysfunction
- Mutations resulting in lysosomal dysfunction may contribute to PD pathogenesis by increasing α-synuclein levels, that in turn may promote aggregation of this protein
- **Hypothesis: Mice heterozygous for loss-of-function PARK9 mutations injected with PFF with have exacerbated pathology and olfactory impairments than their WT littermates**

PARK9 (*ATP13A2*)- *olfaction*









- 1-month WT + PFF mice had an odor detection deficit
- 1- and 3-months post-PBS, *ATP13a2*$^{+/-}$ mice spent less time engaged in investigatory sniffing than WT counterpart
  - heterozygous *ATP13a2* loss-of-function mutations may reduce female's baseline capacity for investigatory sniffing
  - potentially why we failed to see a further decrease for *ATP13a2*$^{+/-}$ females injected with PFF

PARK9 (*ATP13A2*)- *a-synuclein*

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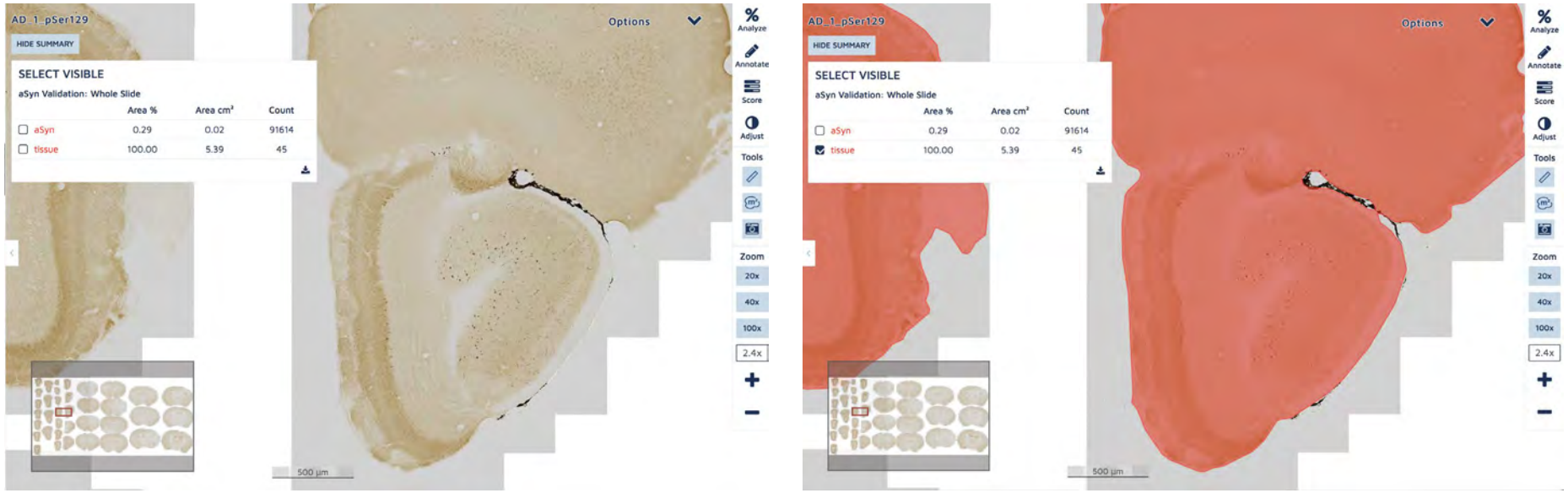
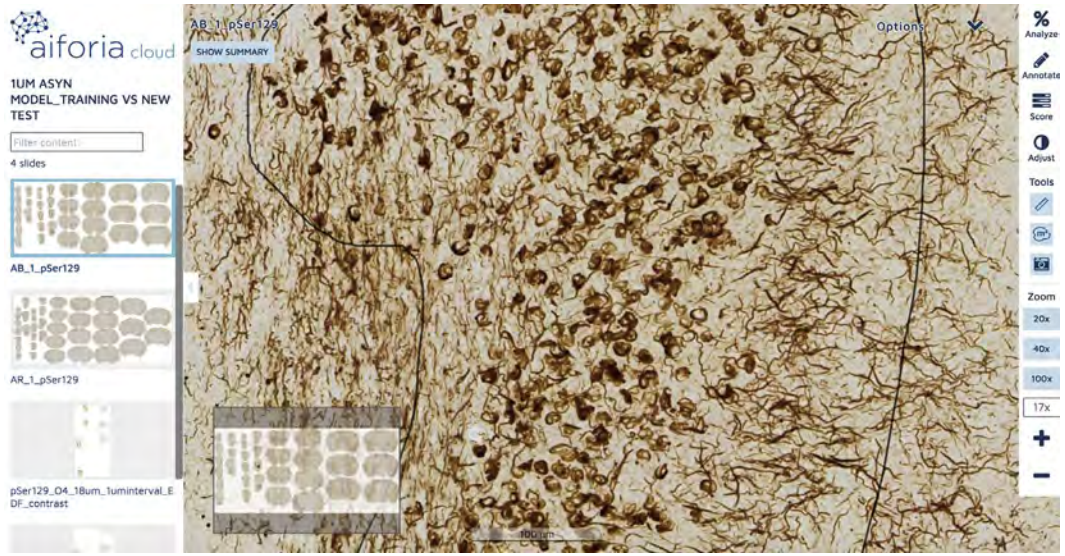
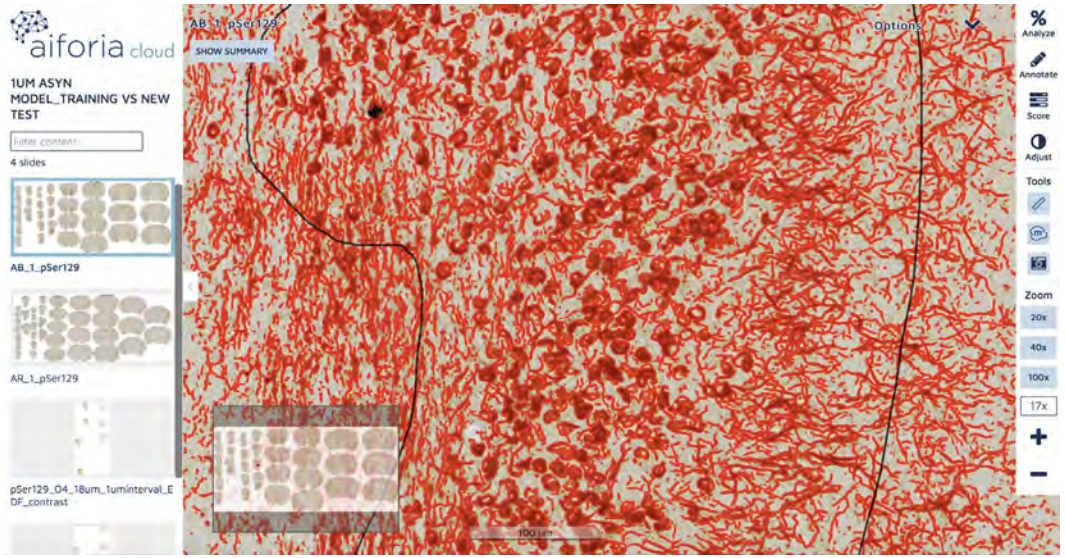
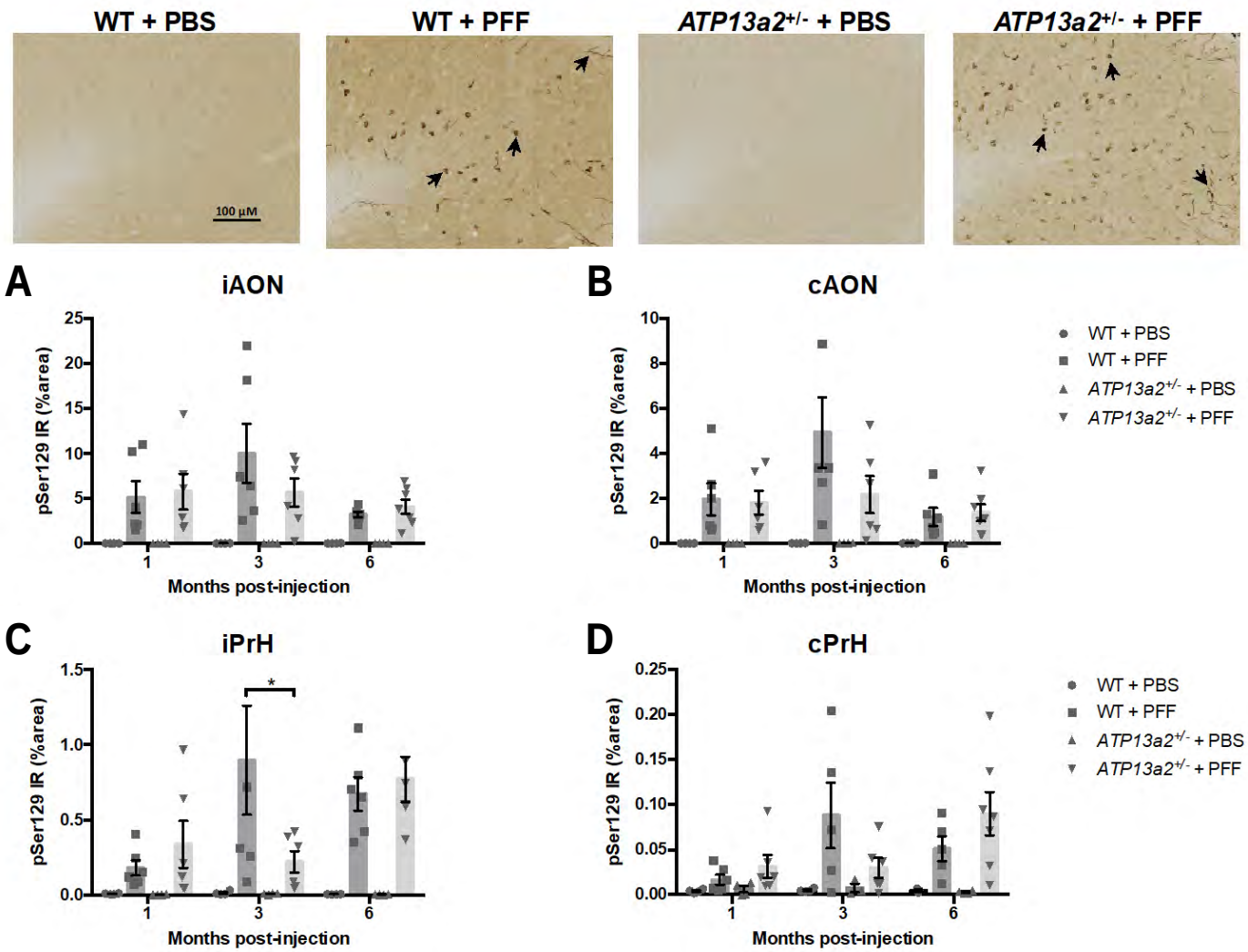
AI for image analysis







PARK9 (*ATP13A2*)- a-synuclein



- 3-months post-PFF, *PARK9* $^{+/-}$ mice had less pathology in the ipsilateral perirhinal cortex (PrH)
- Generally, similar or lower pathology loads in the regions of interest compared to their wildtype littermates
- Previously, *ATP13a2*$^{-/-}$ mice crossed with mice overexpressing the α-synuclein transgene had similar severity of histopathology as the *ATP13a2*$^{-/-}$ only mice (Kett et al., 2015).
- Consistent that the presence of a *ATP13a2* mutation in a model of α-synuclein pathology does not exacerbate pathology development.

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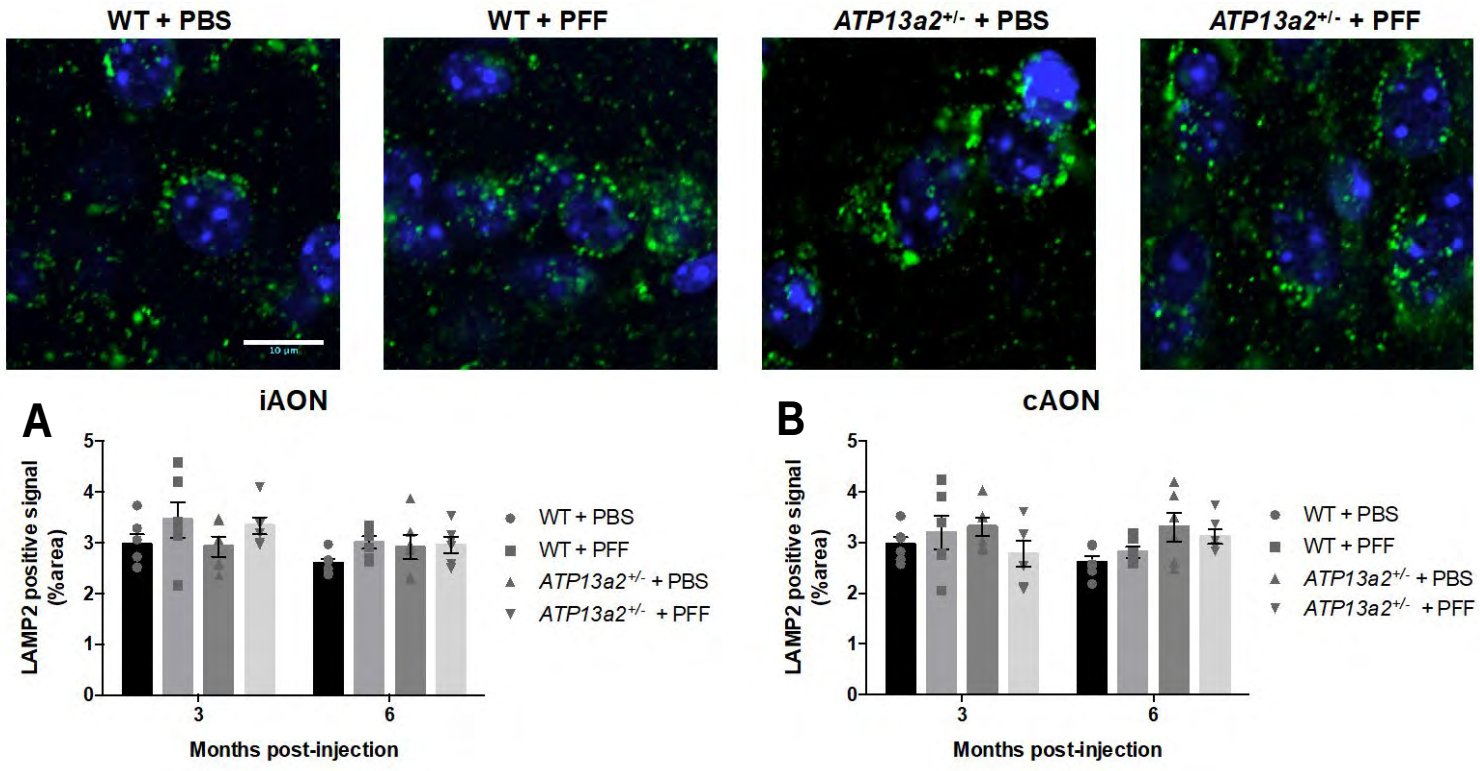
PARK9 (*ATP13A2*)- *LAMP2*



- LAMP2 is one of the lysosome-associated membrane glycoproteins
- Thus, increase in LAMP2 staining is indicative of lysosomal accumulation/increase in the size of lysosomes
- At these ages (6-9 months) LAMP2 levels are unchanged in PARK9 $^{+/-}$ mice regardless of injection type
- Previously, several brain regions of homozygous ATP13a2$^{-/-}$ mice have shown increased levels of LAMP1 and LAMP2 at 18-months of age and increased autofluorescence, indicative of accumulating lipofuscin, at 5-9- and 12-19-months of age.

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Fleming et al., 2018; Kett et al., 2015; Sato et al., 2016; Schultheis et al., 2013

PARK9 (*ATP13A2*)- *Summary*

- Future studies could repeat this experiment using aged mice to investigate whether older mice have more compromised lysosomal function which may result in the heterozygous carriers for loss-of-function *ATP13a2*$^{+/-}$mutations having exacerbated pathology spread compared to their age-matched controls.

- While we did not assess the effect of the mutations in aged or homozygous mice, we postulate that this mutation does not aggravate PD pathology once it has been initiated. Instead, we propose that the mutation acts as a facilitator, upstream in the PD pathogenic process, and promotes the very initial stages of the disease process in the presence of other triggers.

Thank you for listening

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Any questions?